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Sentinel Lymph Node Biopsy: Strategies for Pathologic Examination of the Specimen

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Background and Objectives: Sentinel lymph node biopsy is a practical surgical procedure with high sensitivity for detection of metastases from breast carcinoma in the axilla. It offers economy by avoidance of axillary dissection in the majority of breast carcinoma patients who have negative sentinel node biopsies, and provides an opportunity to study axillary micrometastases with high efficiency in a small volume of tissue.
Methods: Sentinel node tissue is sliced at 2-mm intervals for fixation and paraffin embedding. Probabilities of finding spherical micrometastases of specific sizes randomly distributed in lymph nodes were calculated geometrically for several microsectioning plans.
Results and Conclusions: Sentinel node tissue can studied by systematic serial sectioning technique designed to find metastases of given diameters with specific probabilities. A procedure whereby three microsections are prepared repeatedly at intervals of 250 μm appears to be practical. Two sections from each level can be examined by routine staining and the third by immunohistochemical stain; the latter is recommended particularly for infiltrating lobular carcinoma. This method will find metastases of 0.25-mm diameter with theoretical probability of 1, and metastases of 0.10-mm diameter with probability of 0.46, with reasonable costs. Metastases of these sizes are consequential and worth finding on biological and clinical grounds. *J. Surg. Oncol. 1998;69:212–218.*

KEY WORDS: metastases; sentinel lymph node biopsy; microsectioning

INTRODUCTION

Sentinel lymph node biopsy is a procedure based on the concept that lymphatics from a given site make direct connection with only one or a few lymph nodes, and that metastasis from a tumor will appear first in these nodes. Injection of soluble dye or particulate matter into the tumor will demonstrate the lymph node or nodes that primarily drain the tumor. Among the nontoxic dyes available for this purpose are lymphazurin blue (isosulfan blue) and fluorescein. Lymph nodes remain stained by these two dyes for 30–60 min. Methylene blue staining persists for up to 6 h, but methylene blue is contraindicated in patients with glucose 6-phosphatase deficiency or unstable hemoglobins, and it may be toxic to epithelial cells [1]. Technetium-99m-labeled sulfur colloid or colloidal albumin is useful for localization of sentinel nodes by scintigraphic imaging or use of a handheld γ-counter [2]. The half-life of the metastable Tc-99 isotope is 6.01 h. It decays by isomeric transition with γ-ray emission into technetium 99, a β-emitter with a half-life of 213,000 years. Technetium can be injected ½–3 h before scintiscanning of lymph node sites [3,4]. The technetium method is more sensitive than dye, which may detect only two-thirds of nodes detected by γ emission after technetium uptake [5]. Gulec and associates [6] have reviewed the anatomic and physiologic rationale of sentinel lymph node biopsy and note that a multicenter clinical trial is under way.

Micrometastases may be more readily found in sentinel nodes than in standard axillary dissections, but this impression could be a result of more extensive search of

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TABLE I. Sentinel Lymph Node Biopsy for Breast Carcinoma

Lead author (reference citation)	Method[a]	N[b]	Succ[c] %	Pos[d] %	Sens[e] %	Spec[f] %	Node examination	
							Sectioning[g]	IHC[h]
Krag et al. [2]	TcS	157	76	34.5	95.1	44	1 or 2 sections	No
Pijpers et al. [3]	TcA	37	92	32	100	36	ND[m]	No
Veronesi et al. [4]	TcA	163	98	52	97.5	62	Seven levels	No[l]
Giuliano et al. [7][i]	Blue dye	162	100	42	ND[m]	ND[m]	1 or 2 sections	Keratin 1 sect.
Turner et al. [8]	Blue dye	103	ND[m]	42	99	ND[m]	Three levels	Keratin
Albertini et al. [26]	TcS + blue dye	62	92	32	100 (67)	33	ND[m]	No
Dale et al. [27]	Blue dye	21	66	25	100	40	ND[m]	No
Giuliano et al. [28][i]	Blue dye	174	65.5	32.4	96	62	ND[m]	False-neg.[j]
Giuliano et al. [29][i]	Blue dye	107	93.5		100	33	1 or 2 sections	Keratin
Guenther et al. [30]	Blue dye	145	71	19	97	57	Five levels	No
Meijer et al. [31]	TcA	28	100	32	100	33	ND[k]	ND[k]
Nieweg et al. [32]	Blue dye	22	86	53	100	40	ND[k]	ND[k]
Papa et al. [33]	Vital dye	48	95	ND[k]	91	90	ND[k]	ND[k]

[a]Method for localizing sentinel nodes. TcS, technecium 99m sulfur colloid; TcA, Tc albumin colloid; blue dye, isosulfan blue (Nieweg et al. used patent blue).
[b]No. of patients studied.
[c]Success rate in localizing sentinel node(s).
[d]Prevalence of axillary metastasis.
[e]Sensitivity for detection of axillary metastasis in sentinel node(s).
[f]Prevalence of metastases, one or more, in axillary nodes other than sentinel node(s).
[g]Plan for microsectioning of sentinel node(s).
[h]Immunohistochemical studies.
[i]These reports are from the same institution: patient populations may overlap.
[j]Keratin stain only for false-negative sentinal nodes.
[k]Information from abstract; full text paper not available.
[l]Last 107 patients were examined by bisecting sentinel node(s), preparing three frozen sections from each half, and an unstated number of sections from remnant after fixation and paraffin embedding.
[m]ND, not described.

sentinel nodes. Guiliano et al. [7] found only micrometastasis in 26 of 68 (38%) of patients with sentinel node biopsy plus axillary dissection versus 4 of 39 (10%) of axillary dissections without sentinel node biopsy. Investigators listed in Table I reported finding 1.2–2.2 sentinel nodes per patient on the average. As many as eight have been found in a given patient [8]. Pathologic examination has included keratin staining in some studies (Table I). Precise details of pathologic examination often are lacking in published reports, and we have found no analysis of probability of finding metastases of various sizes.

The rationale for sentinel node biopsy includes avoidance of axillary dissection when metastases are not present, cost-saving, and increased sensitivity of detection of lymph node metastasis. High sensitivity of sentinel node biopsy for detection of axillary metastasis justifies avoidance of axillary dissection when the sentinel node is negative. Alizraki et al. [9] have estimated that sentinel node examination can save about 80% of lymph node staging costs and the morbidity of unnecessary node dissection. Very thorough examination of the sentinel node biopsy is feasible using subserial sectioning because the entire specimen usually can be placed in one or two paraffin blocks.

METHODS AND RESULTS

Geometric Analysis

The simplest model presumes that a lymph node contains only one metastasis, and that the metastasis is spherical. Assuming that metastases form in lymph nodes only if a malignant cell or a group of cells arrive within the node, if the metastasis is in the node, its center will be in the node. The center can be anywhere in the node. The node may be embedded intact or sliced into two or more segments. If the slices are 2 mm thick, they will be embedded *en face*. The center of a single metastasis will be in one of the slices. Probability of intercepting the metastasis in a microsection can be calculated if the placement of the metastasis within the lymph node is random and the metastasis is spherical. For example, if the thickness is broken up into regular intervals of microsections (three totaling 0.015 mm) and unstudied areas (a ribbon of 47 consecutive discarded sections, total = 0.235 mm), eight such intervals will fit into a 2-mm-thick slice (Fig. 1). The studied thickness of the slice will be 8×0.015 mm = 0.120 mm, and the unstudied thickness will be 4×0.235 mm = 1.880 mm. The latter will be broken up into eight segments of 0.235 mm. A 0.1-mm spherical

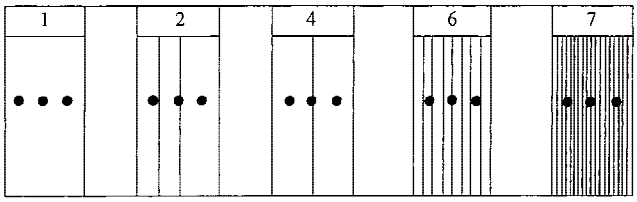

Fig. 1. Diagram of plans 1, 2, 4, 6, and 7. Tissue blocks are represented on edge at one-fourth height relative to thickness. Scale 13:1; actual block thickness is 2 mm. Dots, metastatic tumors 0.2 mm in diameter. If a tumor does not fall on a vertical line representing a microsection, it remains undetected.

tumor can be placed with the center anywhere within a zone 0.05 mm of either end of the 0.235-mm segment, leaving a span of 0.135 mm in which it can be placed without being included in a microsection. Counting the three 5-μm sections that have been subtracted, the total possible span in which the center of the sphere could fall is 0.25 mm. Given random placement of the sphere, the probability of not encountering it in a section will be 0.135/0.250 = 0.54, and the probability of finding it will be 0.46. Equation (1) expresses the probability of finding metastases of diameters less than the unstudied interval for various combinations of sampling intervals and numbers of sections.

$$P = 1 - [\text{Tint} - \{(\text{Tms} \cdot \text{Nms}) + \text{Dt}\}]/\text{Tint} \qquad (1)$$

where *P* is the probability of finding the tumor in a microsection, Tint is thickness of the interval into which the slice is divided by the repetitive procedure (0.250 mm in the example above), Tms is thickness of the microsection (0.005 mm), Nms is the number of microsections in each interval, and Dt is the diameter of the spherical tumor. When the diameter of the tumor exceeds the unstudied interval (0.135 mm in the example above), it will always be discovered. On the basis of these considerations, probabilities of detection of spherical metastases of different diameters are listed for several illustrative plans (Tables II, III). When residual block thickness exceeds the sectioning interval, as in plan 2, probability of finding the tumor is the mean of the summed probabilities of finding it if in the sectioned part of the block, using equation (1), or unsectioned part of the block, where the probability of finding it is zero.

Biologic Considerations Regarding Micrometastases

Known rates of proliferation and growth of breast carcinomas provide insight into the duration of metastases of a given size in lymph nodes. Equation (2) expresses relationships between volume of the tumor, growth constant, and the number of generations

$$\frac{V}{V_0} = e^{kt} \qquad (2)$$

where V is the volume when measured at time t, V_0 is volume at time zero, and e is the base of natural logarithms. Time t is measured in units equivalent to cell cycle duration. If t is set to 1 and V/V_0 to the increment in volume during one cell cycle duration, the equation can be solved for the growth constant k by taking logarithms. If the tumor actually doubles during one cell cycle (no cell loss), $V/V_0 = 2$ and $k = 0.693$, the natural logarithm of 2. If one-half of the cells generated during a cell cycle are lost, $V/V_0 = 1.5$ and $k = 0.405$. If 90% of cells are lost, $V/V_0 = 1.1$ and $k = 0.095$. Under the latter circumstance, cell proliferation is largely ineffective, and despite rapid cell division growth is slow. The number of generations necessary to reach a given volume which contains a given number of cells will depend on rate of cell loss (Table IV). For example, if cell loss factor is zero, a diameter of 10 mm could be achieved in 28 generations. But if cell loss factor is 0.9, 205 generations would be required. The latter circumstance would give the tumor much more opportunity for genetic evolution than the former because of the larger number of cell divisions required to reach detectable size.

Comparison of potential doubling time (mean cell cycle time) [10,11] of breast carcinoma with measured growth rates [12] shows that mean cell loss actually averages 90% or more during the clinically detectable phase of disease. Measurements of S-phase fraction of 450 infiltrating breast carcinomas by bromodeoxyuridine labeling of tissue slices showed the mean to be 6.4%, median 3.9% [10]. This is equivalent to a median cell cycle time of 14 days according to Steel's formula (equation 3) if the median S-phase duration is 18 h [13]. Goodson and associates recorded slightly higher results with in vivo bromodeoxyuridine labeling of relatively large breast carcinomas [14]. The difference is accounted by increase of mean S-phase fraction with increasing tumor size [10]. Actual measured growth rates of breast carcinomas by Spratt and associates indicate a median doubling time of 260 days for 448 patients at diagnosis [12]. The large disparity between cell cycle time, which is equivalent to potential tumor doubling time, and measured doubling time indicates a high rate of cell loss from breast carcinoma. Inserting the median values into Steel's formula for cell loss [13] would yield a cell loss rate of 95%. High rates of cell loss seem rational when considered in light of rapid proliferative rates of many breast carcinomas and inherently slow proliferative rate of stromal tissues and their vascular support, which are dependent on cytokines produced by the breast carcinoma cells [15,16]. Evidence for deceleratory growth of breast carcinoma has been cited [12], although its actual demonstration by direct measurement is lacking. Thus, rate of cell loss may be lower when the neoplasm is small, but long latency of breast carcinoma metastasis before clinical presentation argues against more rapid

TABLE II. Several Plans for Examination of Sentinel Nodes

Plan	Description	Cycle length (mm)	No. of cycles	No. of microsections		Discarded ribbon length, sections (mm)	Residual tissue (mm)
				H&E[a]	Immuno[b]		
1	One level	2	1	2	1	0	1.980
2	Three levels	0.5	2	4	2	96	0.985
3	Two levels	1	2	4	2	196	0.985
4	Three levels	1	3	6	3	197	0.000
5	Four levels	0.5	4	8	4	97	0.485
6	Eight levels	0.25	8	16	8	47	0.235
7	Sixteen levels	0.125	16	32	16	22	0.110

[a]H&E, hematoxylin and eosin.
[b]Immuno, immunostaining.

TABLE III. Probability of Finding Tumor When Node Is Sliced at 2-mm Intervals and Totally Embedded for Microsectioning

Tumor diameter (mm)	Probability of detecting tumor						
	Plan 1	Plan 2	Plan 3	Plan 4	Plan 5	Plan 6	Plan 7
2.0	1	1	1	1	1	1	1
1.0	0.51	1	1	1	1	1	1
0.50	0.26	0.76	0.51	0.52	1	1	1
0.25	0.12	0.40	0.26	0.27	0.53	1	1
0.20	0.11	0.32	0.23	0.23	0.43	0.86	1
0.15	0.08	0.25	0.16	0.17	0.33	0.66	1
0.10	0.06	0.17	0.11	0.12	0.23	0.46	0.92
0.075	0.05	0.13	0.09	0.10	0.18	0.36	0.72
0.05	0.03	0.10	0.06	0.07	0.13	0.26	0.52
0.025	0.02	0.06	0.04	0.05	0.08	0.16	0.32
0.015	0.02	0.04	0.03	0.04	0.06	0.12	0.24
0.01	0.01	0.04	0.03	0.03	0.05	0.08	0.20

growth of micrometastases. Otherwise relapse years and even decades after initial treatment could not be explained other than by invoking the mysterious concept of dormancy [17]). For purpose of modeling (*vide infra*), we chose to include cell loss rates that are actually much lower than directly measured in breast carcinomas because of uncertainty about these rates in tumors of subclinical sizes.

If one accepts the concept that cell loss rates are high from the initial stages of breast carcinoma metastatic development, then the number of generations required for the metastases to reach even small size is impressive and argues that these metastases have been present for months, even years. Beginning with the paper by Saphir and Amromin [18], retrospective studies in which lymph nodes have been subserially sectioned have shown a rate of metastasis close to 20% in axillae deemed negative from the results of routine histologic study [19]. Early studies characterized by small numbers of patients and short followup commonly failed to demonstrate the prognostic effect of micrometastases. Dowlatshahi and associates [19] reviewed a large number of studies and found that significant survival differences commonly were demonstrated in studies with at least 147 subjects (nine studies) and follow-up of at least 6 years (one additional study). Within the context of a randomized therapeutic trial to which 921 node-negative patients were accessioned, micrometastases were associated with decreased relapse-free survival ($P = 0.0008$) and decreased survival ($P = 0.0009$) at 6 years of observation [20,21]. Intuitively, metastases from small carcinomas are likely to be small in comparison to those from large primary carcinomas, making detection more difficult. Low probability of detecting small lymph nodal metastasis by traditional examination of one section of each lymph node raises doubt about reported low rates of positive axillary nodes related to small breast carcinomas [22]. If even small tumor deposits have high rates of cell loss, metastases could grow slowly enough to remain undetectable by conventional techniques for prolonged periods. Table IV lists the number of cell generation times necessary for tumors to reach a given size when differing rates of cell loss apply. Time elapsed from one mitosis to the next is a generation. It can be approximately calculated from Steel's formula if the DNA labeling index (LI) and duration of DNA synthesis (Ts) are known. The LI is the proportion of nuclei in S-phase measured by uptake of tritiated thymidine or bromodeoxyuridine:

$$T_G = k \frac{T_s}{LI} \tag{3}$$

where K is a constant accounting for the position of DNA synthesis in the cell cycle of an exponentially growing population and a reasonable estimate is 0.75 [13]. A reasonable approximation of Ts is 18 h from studies on a variety of human cancers [11]. Growth rates have not been measured directly for human tumors of subclinical size. Measurement of serum prostate-specific antigen (PSA) has shown long doubling times for subclinical metastases similar to doubling times measured for clinically evident prostate cancer [23], suggesting that, at least for this type of cancer, small lesions do not possess

TABLE IV. Number of Cells and Generations by Different Tumor Sizes

Diameter (mm)	Volume (mm^3)	No. of cells	No. of generations from one cell with different rates of cell loss		
			Cell loss = 0	Cell loss = 0.5	Cell loss = 0.9
10	524	296×10^6	28.1	48.1	205
5	65.4	37×10^6	25.1	43	183
2	4.18	2.4×10^6	21.2	36.2	155
1	0.52	294×10^3	18.2	31.1	136
0.5	0.065	37×10^3	15.2	25.9	111
0.25	0.0082	4,629	12.2	20.8	89
0.2	0.0042	2,370	11.2	19.2	82
0.1	0.00052	296	8.2	14	60
0.05	65×10^{-6}	37	5.2	8.9	38
0.025	8.2×10^{-6}	5	2.3	3.9	17
0.015	1.8×10^{-6}	1	0	0	0

more capacity for rapid growth than do large lesions. It may then be of some interest to consider how long metastatic tumors of various sizes have been present given an assumption of constant cell generation time and constant rate of cell loss over time (Table V). Given a cell T_G of 15 days and cell loss factor of 0.9, which are reasonable values for breast carcinoma based on available studies, a 0.1-mm-diameter metastasis would have been present for approximately 2.4 years. Even with an extremely low cell loss factor of zero, it would have been present for 4 months and a 0.5-mm metastasis for nearly 8 months.

DISCUSSION

We appear to be at the threshold of an era in which sentinel lymph node biopsy largely will replace node clearance dissection for cancer staging. The studies reviewed above indicate that this technique is highly sensitive for selection of lymph nodes bearing metastases in the axilla, and similar results have been obtained for malignant melanoma [5,24,25]. By detection and selective biopsy of nodes in the primary lymphatic drainage of tumors, we have opportunity for intensive study of micrometastases and their significance in prognosis and therapy. To grasp this opportunity, we need to examine the biopsy specimens by systematic techniques that provide known power for detection of metastases of different sizes. This requires a disciplined approach in the pathology laboratory. Power of detection should be balanced against cost, but clearly one or two hematoxylin and eosin (H&E)-stained sections are inadequate for examination of sentinel nodes.

The analysis presented in this paper suggests that procedures can be designed that will find metastases of designated sizes with definable probabilities. Our treatment assumes random distribution of spherical metastases within lymph nodes. Neither of these assumptions is entirely correct. Metastases tend to form in the capsule or subcapsular regions of lymph nodes. However, using this knowledge to design histopathological approaches presents spatial difficulties, and we have not attempted to address this consideration. Shape is dependent on constraints of surrounding tissue and microenvironmental conditions influencing growth. In addition, progression of genetic abnormalities could lead to growth rate changes focally in a metastasis with resultant distortion of shape. For these reasons, the calculations presented in this paper are approximations, but they are useful in developing rational designs for histopathologic examination.

The most cost-effective approach will be stepwise. Lymph nodes with grossly visible metastases do not require special treatment. If grossly negative, the first step would be one or two microsections from the face of the block. If negative for metastasis, a systematic serial sectioning approach will follow. The procedure to be chosen can be based on the minimum size metastasis one wishes to detect with a high degree of probability. Currently no data exist to specify what size this should be. Therefore choice may be driven chiefly by considerations of labor and economics. A 0.25-mm-diameter metastasis can be found with theoretical certainty by plan 6 (Tables II, III), which requires examination of a minimum of 16 microsections (or 8 if only one section at each level is studied). This procedure would identify nearly one-half of metastases 0.10 mm in diameter and would appear to be practical. The number of microsections prepared by the tissue laboratory would be comparable to mastectomy and axillary node dissection or radical prostatectomy specimens, for example, and viewing time by the pathologist would also be comparable to these specimens. Plan 7, requiring examination of at least 32 (or minimally 16) microsections, would have power to detect virtually all metastases of 0.15-mm diameter, but would probably be too tedious for routine application. Either plan could be truncated pending examination of microsections from initial levels, and deeper levels could be avoided if the tumor is found. However, in order to record more accurate dimensions of metastases, the serial sectioning procedure should be carried out to completion. Addition of immunohistochemical examination would entail consid-

TABLE V. Estimated Duration of Metastasis by Size, T_{Dpot}, Cell Loss

	Duration of metastasis in years dependent on cell loss factor and generation time (T_G)								
	Cell loss = 0			Cell loss = 0.5			Cell loss = 0.9		
Diameter	T_G, days			T_G, days			T_G, days		
(mm)	3	15	55	3	15	55	3	15	55
10	0.23	1.15	4.23	0.40	1.98	7.25	1.69	8.42	30.1
5	0.21	1.03	3.78	0.35	1.77	6.48	1.50	7.52	27.6
2	0.17	0.87	3.2	0.30	5.45	5.45	1.27	6.37	23.4
1	0.15	0.75	2.74	0.26	12.28	4.69	1.12	5.59	20.5
0.5	0.12	0.62	2.29	0.21	1.06	3.9	0.91	4.56	16.7
0.25	0.10	0.5	1.83	0.17	0.85	3.13	0.73	3.65	13.4
0.2	0.09	0.46	1.67	0.16	0.79	2.89	0.67	3.37	12.4
0.1	0.07	0.34	1.23	0.12	0.58	2.11	0.49	2.47	9.04
0.05	0.04	0.21	0.78	0.07	0.37	1.34	0.31	1.56	5.73
0.025	0.02	0.09	0.35	0.03	0.16	0.59	0.14	0.7	2.56
0.015	0	0	0	0	0	0	0	0	0

erable increases in expenditure. No consensus on the role of immunohistochemistry in identifying metastasis has been reached [19]. We believe it should be used routinely when the primary lesion is infiltrating lobular carcinoma which can produce metastases difficult to detect by routine examination. An estimate of histotechnologist's time for the sectioning procedure in plan 6 is 15 min per block. Total costs of thorough systematic study of sentinel nodes should be seen against a perspective of the important light it can shed on breast carcinoma behavior when the metastasizing phenotype is revealed in the microsection. Appropriate reimbursement codes for this type of sentinel node examination have not yet been devised.

Systematic subserial sectioning sentinel node examination can be likened to transaxial computerized imaging of the liver wherein the organ is visualized not by one or two capriciously selected cuts, but at regular intervals of known width. This model is a necessary one for pathologists to follow in order to control power of detection of metastases and optimize the histopathologic examination.

REFERENCES

1. Jasinski RW, Smith DC, Chase DR, Field FI: Angiographic preoperative bowel segment localization using methylene blue, isosulfan flue, and fluorescein. Invest Radiol 1987;22:462–466.
2. Krag DN, Ashikaga T, Harlow SP, Weaver D: Development of sentinel node targeting technique in breast cancer patients. Breast J 1998;4:67–74.
3. Pijpers R, Meijer S, Hoekstra OS, et al.: Impact of lymphoscintigraphy on sentinel node identification with technetium-99m-colloidal albumin in breast cancer. J Nucl Med 1997;38:366–368.
4. Veronesi U, Paganelli G, Galimberti V, et al.: Sentinel-node biopsy to avoid axillary dissection in breast cancer with clinically negative lymph-nodes. Lancet 1997;349:1864–1867.
5. Wells KE, Rapaport DP, Cruse CW, et al.: Sentinel lymph node biopsy in melanoma of the head and neck. Plastic Reconstr Surg 1997;100:591–594.
6. Gulec SA, Moffat FL, Carroll RG, Krag DN: Gamma probe guided sentinel node biopsy in breast cancer. Q J Nucl Med 1997; 41:251–261.
7. Giuliano AE, Dale PS, Turner RR, et al.: Improved axillary staging of breast cancer with sentinel lymphadenectomy. Ann Surg 1995;222:394–399.
8. Turner RR, Ollila DW, Krasne DL, Giuliano AD: Histopathologic validation of the sentinel lymph node hypothesis for breast carcinoma. Ann Surg 1997;226:271–276.
9. Alazraki NP, Eshima D, Eshima LA, et al.: Lymphoscintigraphy, the sentinel node concept, and intraoperative gamma probe in melanoma, breast cancer, and other potential cancers. Semin Nucl Med 1997;27:55–67.
10. Meyer JS, Koehm SL, Hughes JM, et al.: Bromodeoxyuridine labeling for S-phase measurement in breast carcinoma. Cancer 1993;71:3531–3540.
11. Wilson GD, McNally NJ, Dische S, et al.: Measurement of cell kinetics in human tumours in vivo using bromodeoxyuridine incorporation and flow cytometry. Br J Cancer 1988;58:423–431.
12. Spratt JA, von Fournier D, Spratt JS, Weber EE: Deceleratory growth and human breast cancer. Cancer 1993;71:2013–2019.
13. Steel GG: ''Growth Kinetics of Tumours.'' Oxford: Clarendon Press, 1977:66–75.
14. Goodson WH, Ljung BM, Waldman FM, et al.: In vivo measurement of breast cancer growth rate. Arch Surg 1991;126:1220–1223.
15. Folkman J: The influence of angiogenesis research on man agement of patients with breast cancer. Breast Cancer Res Treat 1995; 36:109–118.
16. Guidi AJ, Schnitt SJ, Fischer L et al.: Vascular permeability factor (vascular endothelial growth factor) expression and angiogenesis in patients with ductal carcinoma in situ of the breast. Cancer 1997;80:1945–1953.
17. Brinkley D, Haybittle JL: The curability of breast cancer. Lancet 1975;2:95–97.
18. Saphir O, Amromin GD: Obscure axillary lymph node metastases in carcinoma of the breast. Cancer 1948;1:238–241.
19. Dowlatshahi K, Fan M, Snider HC, Habib FA: Lymph node metastases from breast carcinoma. Reviewing the dilemma. Cancer 1997;80:1188–1197.
20. Bettelheim R, Price KN, Gelber RD, et al., for the International Breast Carcinoma Study Group: Prognostic importance of occult axillary lymph node micrometastases from breast cancers. Lancet, 1990;335:1565–1568.
21. Neville AM, Price K, Gelber RD, Goldhirsch A: Axillary node micrometastases and breast cancer. Lancet 1991;337:1110.
22. Silverstein MJ, Gierson ED, Waisman JR, et al.: Axillary lymph node dissectin for T1a breast carcinoma. Is it indicated? Cancer 1994;73:664–777.
23. McLaren DB, McKenzie M, Duncan G, Pickles T: Prostate specific antigen doubling times and clinical behavior in patients with early untreated prostate carcinoma. Cancer 1998;82:342–348.
24. Glass LF, Messina JL, Cruse W, et al.: The use of intraoperative

radiolymphoscintigraphy for sentinel node biopsy in patients with malignant melanoma. Derm Surg 1996;22:715–720.
25. Leong SP, Steinmetz I, Habib FA, et al.: Optimal elective sentinel lymph node dissection in primary malignant melanoma. Arch Surg 1997;132:666–672.
26. Albertini JJ, Lyman GH, Cox C, et al.: Lymphatic mapping and sentinel node biopsy in the patient with breast cancer. JAMA 1996;276:1818–1822.
27. Dale PS, Williams JT IV: Axillary staging utilizing selective sentinel lymphadenectomy for patients with invasive breast carcinoma. Am Surg 1998;64:28–31.
28. Giuliano AE, Kirgan DM, Guenther JM, Morton DL: Lymphatic mapping and sentinel lymphadenectomy for breast cancer. Ann Surg 1994; 220:391–398.
29. Giuliano AE, Jones RC, Brennan M, Statman R: Sentinel lymphadenectomy in breast cancer. J Clin Oncol 1997;15:2345–2350.
30. Guenther JM, Krishnamoorthy M, Tan LR: Sentinel lymphadenectomy for breast cancer in a community managed care setting. Cancer J Sci Am 1997;3:336–340.
31. Meijer S, Collet GJ, Pijpers HJ, et al.: Less axillary dissection due to sentinel node biopsy in patients with breast carcinoma. Nederl Tijdschr Geneesk 1996;140:2239–2243.
32. Nieweg OE, Kapteijn BA, Peterse JL, et al.: Identification of the sentinel node in patients with breast carcinoma. Nederl Tijdschrift Geneesk 1996;140:2235–2239.
33. Papa MZ, Bersuk D, Koler M, et al:. Identification of sentinel and axillary node involvement in breast cancer. Harefuah 1997;133: 428–430.